REMARKS

Claim 32 has been amended to delete "in which organism it is suspected that PTGS is occurring." This phrase was objected to as not appearing *in haec verba* in the specification, and although applicants believe it would be unlikely to carry out the method if silencing were not suspected, it appears that this phrase does not add anything to the claim and therefore it is deleted. This disposes of the new matter rejection.

Claim 32 has also been amended to require that the short RNA molecules be of uniform length to indicate the occurrence of silencing. Support for this requirement is found, for example, on page 22, lines 6-7. This amendment has been made to allay the apparent concerns of the Office that degradation products as experimental artifacts might cloud the results.

Claim 32 has been amended as well to include the suggestion kindly made by Examiner Schultz at the interview to require a step that the silencing of the target gene be confirmed. Support for this addition is found, for example, on page 3, last two lines. While the word in the specification is "correlated" it is believed that "confirm" is more clear.

Finally, claim 32 has been amended to recite "gene silencing" in general, rather than specifying a particular mechanism – *i.e.*, post-transcriptional gene silencing. The specification supports this general term, for example, in the Abstract, the Title, the description of the Technical Field, and in the description at page 9, at lines 6-20 which discusses, in general, the claimed method, and on pages 9-10, bridging paragraph.

Also, claims 39-40 which were withdrawn from consideration have been canceled.

New claims 68 and 69 are simply narrower forms of claims 32 and 66.

Support for new claim 70 is found on page 9, lines 6-14.

New claims 71-81 merely recite, on a claim-by-claim basis, the individual sizes of SRMs within the 20-30 nt range recited in claim 32 and generically supported in the disclosure (about 25 nt in length, 25 nt plus minus 5 nucleotides – see page 4, lines 4-14 of the application, etc.).

New claims 82 and 83 are proposed in the event that the uncertainty asserted to be associated with the method of claim 32 resides solely in the possibility that "or similarity" is included, rather than only absolute identity. Thus, the step of confirming gene silencing has been moved to a dependent claim. *Should claims 82 and 83 be the only claims standing in the way of an allowance, cancellation of these claims by Examiner's amendment is respectfully requested.*

These amendments do not introduce new matter.

Again, applicants express their appreciation with respect to withdrawal of the previously made rejections and note that the new matter rejection on pages 2-4 of the Office action is now moot.

The Pending Rejection

Only one outstanding rejection remains, and applicants believe this has been addressed by the amendment to claim 32 and by new claims 82 and 83. In the discussion below, the manner in which this amendment remedies any defects asserted by the Office in the rejection is detailed.

All examined claims were rejected under 35 U.S.C. § 112, first paragraph, as assertedly lacking enablement as to their full scope. As discussed at the interview, the scope problem seems to arise because a specific degree of homology representing similarity is not specified, and therefore it is unclear which, if any, gene is being silenced. By requiring a step to confirm that the target gene is silenced, or alternatively by requiring sequence identity, this uncertainty is resolved.

In general, the Office states that the specification "does not reasonably provide enablement for a method of detecting PTGS of *any* target gene in *any* mammal via the detection of *any* SRM with *any* degree of identity or similarity with a target gene." (Emphasis added.) To clear the air, most if not all of the "any's" have nothing to do with claim scope. The claims do not require that any arbitrarily chosen gene be detected; only those that actually are undergoing silencing would be detected in any case. This does not broaden the claim at all. Neither does "any" SRM broaden the claim since either the SRMs are there or they are not. Neither does "any" mammal broaden the scope of the claim, since only those mammals wherein silencing is occurring, as indicated by the detection in such mammals of SRMs, are included within the claim scope. As to "any" degree of identity – identity is identity – there is no scope there. As to "any" degree of similarity – only those SRMs that are present will be detected and related back to genes with sufficient degree of similarity to actually have been silenced. In light of the amendment to the claims, it is clear that only silenced genes will be confirmed to have been detected by detecting similar SRMs.

Applicants acknowledge that no specific cutoff for similarity has been provided; this is because practitioners of the art are sufficiently sophisticated to exercise professional judgment in this regard. The claim merely requires that the presence of SRMs having identity or similarity with a target gene "indicates" silencing of the target gene. Conclusive proof is not required by the characterizing step in the claim. One of skill in the art is enabled by the present disclosure to explore which genes containing sequences identical or similar to the detected SRMs are actually being silenced by looking at, for example, changes in phenotype or reductions in gene products such as proteins and mRNA encoded by genes identified by the claimed method.

This latter step is now specifically required by all claims except 82 which requires sequence identity and thus any errors in identifying the silenced target gene would be remedied by this step, as Examiners Schultz and Bowman kindly acknowledge.

While applicants are grateful that allowability of claim 32 and its dependent claims appears to be acknowledged with the amendment to claim 32 proposed in this response, in order to complete the record, applicants advance further arguments in support of the scope of the claims as now proposed, in the event further concerns remain.

In the outstanding basis for rejection, based on the unamended claim, the Office acknowledges only the nexus between gene silencing and SRMs in the specific examples provided. Respectfully, this is improper. MPEP § § 2164.08 quotes from *In re Goffe*, 542 F2d 564, 567, 191 USPQ 429, 431 (CCPA 1976) for the proposition that this is unfair.

In *Goffe*, the invention was directed to a method of obtaining images on an imaging plate which comprised a support on which there was a non-gaseous agglomeratable layer composed of particles that would agglomerate in the presence of heat. Thus by applying heat selectively only to portions of the agglomeratable layer, an image could be obtained. The claim did not specify any materials that would be included in the agglomeratable layer and the specification mentioned only three. The Court reversed the rejection of the claim made on the basis of scope, as it was clear that practitioners of the art would not use any of the obviously inappropriate materials suggested by the PTO that presumably would be included within the scope of the claim. In articulating the policy behind this, the Court stated

> To provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for "preferred" materials in a process such as the one herein

> involved would not serve the Constitutional purpose of promoting progress in the useful arts.

As further noted by the Court, if only the named materials were covered by the scope of the claim, all a competitor would need to do is to substitute some clearly similar materials and the inventor would be deprived of the benefits of the advance he had made in the art.

Similarly, here, all a competitor would need to do is apply the methods that have been demonstrated in plants to other organisms and the contribution to the art of the present inventors would be subverted.

This statement of policy further confirms that the claims should not be limited to the postulated mechanism of silencing as the essential discovery of applicants is that gene silencing is associated with short RNA molecules, not the mechanism therefor.

In addition, the present application itself makes clear that the invention extends beyond the exemplified plants and nematodes. As stated on page 3:

> Importantly, the disclosure herein provides evidence that SRMs may be a common mediator of PTGS in both plants and higher organisms such as the nematode discussed in the examples hereinafter.

In addition, claim 1 as originally filed clearly covers organisms other than plants as evidenced by dependent claims 3-4 (which specified nematodes and mammals respectively). These claims are, of course, considered part of the application as originally filed. Thus, it is clear that the specification teaches that this method is applicable to systems other than plants, including mammals specifically.

The skilled artisan at the time of the invention in 1999 would have had no reason to doubt statements in the specification that the presence of SRMs is indicative of silencing in organisms

generally, particularly where, as in claim 32 as herein amended, the SRMs are of uniform length, as opposed to, for example, random RNA degradation products which might be present as artifacts in the extract due to the isolation procedure itself.

Many later-published documents confirm the applicability of this invention to detection of silencing in various organisms, including mammals. See, for example, Babiarz, *et al., Genes & Development* (2008) 22:2773-2785, "Mouse ES cells Express Endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent Small RNAs"; Watanabe, *et al., Nature* (2008) 453:539-544, "Endogenous siRNAs from Naturally Formed dsRNAs Regulate Transcripts in Mouse Oocytes"; Sontheimer and Carthey, *Cell* (2005) 122:9-12, "Silence from within: Endogenous siRNAs and miRNAs".

Applicants understand that enablement is evaluated at the time of the invention, but this means that at the time of the invention there must be some reason to doubt the statements made in the specification that SRMs would be detectable in mammals where silencing is occurring. There is no such reason provided, and the later-published documents cited above confirm that applicants' statements are correct.

That said, it is believed that even if the foregoing argument is not accepted, it is inapplicable to the claim 32 as now amended, since the required confirmation of silencing step is present.

The citation of Elbashir, *EMBO J.* (2001) 20:6877-6888 does not provide any reason to question the scope of the claims. Elbashir is concerned with studies that "provide a rational basis for the design of siRNA's in future gene targeting experiments", (see Abstract), rather than with detecting, empirically, which SRMs are present when silencing is occurring and what the sequence of such detected SRMs are. Elbashir employs an artificial system – *Drosophila* embryo lysates – to

determine the most efficient configurations that might be supplied to the lysate in order to effect cleavage of target RNA. This is only tangentially related to the ability of the practitioner to carry out the methods described in the present invention, which are directed to detection of silencing already occurring, not effecting it.

The distinctions between that which is disclosed by Elbashir and the present invention are embodied in new claim 70, presented herein. While Elbashir tests synthetic candidate SRMs for effectiveness, the present invention relates to an empirical method to determine what target gene is silenced. In the course of such analysis, it is also revealed as set forth in claim 70 which sequences homologous to the target gene are effective at inducing silencing.

As the specification makes clear, detection of short RNA molecules (SRMs) meeting the elements of the present claims is an indication that silencing is occurring as well as an indication of which genes are being silenced (those that are identical or sufficiently similar to base pair with the detected SRMs). The presence, therefore, of SRMs of uniform length in the size range of 20-30 nucleotides is diagnostic. The teaching in Elbashir that "not all positions of the siRNA contribute equally to the target recognition and mismatches in the center of the siRNA duplex prevented target RNA cleavage" is not seen as relevant. If the SRMs are present, it simply follows that a gene with sufficient similarity to or identity with the detected SRM is undergoing silencing. In addition, as exemplified in claim 41, the characterization can be performed by probing a library of genes, and any sequence that binds the SRM would be sufficiently similar to conclude that that gene is indicated as a target gene of the detected SRM. The "any similarity" issue is moot, of course, with respect to new claims 68, 69, and 82.

In any event, since confirmation of silencing of the target gene is required, the judgment of what is similar is validated by that required step.

Perhaps unresolved with respect to even the amended claim is the assertion by the Office that since Elbashir teaches that siRNA molecules are double-stranded molecules, and since the claims do not require detection of both SARMs and SSRMs or require the molecules to be duplexed, this indicates a lack of enablement. Respectfully, this is not correct. Even if the actual silencing mechanism involves duplexed RNA, the RNA's that are isolated may readily be separated and separately detected. It is not necessary to detect both SSRMs and SARMs since the presence of one is indicative of the presence of the other. As the application states, in every case where SARMs are found, corresponding SSRMs are found as well. No reason has been provided to doubt this; and thus the presence of one is indicative of the presence of the other.

In addition, while it is understood that each case stands on its own merits, this issue has already been resolved in connection with the same application by the U.S. PTO in the issuance of the parent application herein as 6,753,139. In this patent, which has an identical specification to the present one, claims were issued directed to detection of silencing in plants by means of detection of SRMs including either SSRMs or SARMs individually (claims 2 and 3). The claims issued in the '139 patent also include analysis of SRMs having sequences that are "identical to or similar to" the sequence of the target gene.

It does not follow from any requirement involved in the silencing process itself that in order to detect the presence of silencing, it would be necessary to detect both SSRMs and SARMs in duplex form. This ignores the results of the isolation procedure taught by the specification itself.

The Office is confusing what is effective to induce silencing with the nature of the evidence that suffices to detect it.

And there is no demonstration or documentation showing that SSRMs or SARMs of uniform length arise independently in other contexts. This is stated explicitly in the last paragraph of Example 1 of the subject application:

> However the 25 nt RNA has never been detected in the absence of PTGS. This correlation and the properties of 25 nt RNA are consistent with a direct role for them in PTGS induced by, for instance, transgenes or viruses (12). 25 nt RNA species also serve as molecular markers for PTGS. Their presence could be used to confirm other examples of, *e.g.*, transgene or virus-induced PTGS and may also serve to identify endogenous genes that are targeted by PTGS in non-transgenic plants.

This was confirmed experimentally when, as set forth in this Example, low molecular weight RNA was extracted from plants containing silencing (S) or non-silencing (NS), 35S-ACC-oxidase (ACO) or 35S-GFP transgenes and hybridized with labeled RNA probes transcribed in the sense orientation from ACO and GFP cDNAs. Single stranded RNA was removed by digestion with RNAaseONE, and any remaining RNA molecules (which are protected by the labeled probes) were denatured and separated by electrophoresis. With the ACO probe, protected fragments seen on the gel are obtained only with RNA from the ACO silencing tomato plants and with the GFP probe only with RNA from the GFP silencing plants. The short RNA species detected in this assay correspond to the 25 nt RNA detected by Northern analysis as described before in this example. (They are a bit more disperse because of RNAase digestion at the ends of breathing RNA duplexes, and some higher molecular weight signals were also obtained, possibly as a result of incomplete digestion of single stranded regions.) This experiment directly demonstrates the sequence specificity of the diagnostic SRM and that their appearance occurs only during silencing.

The attached publication by Overhoff, *et al., Nucleic Acids Research* (2004) 32:e170 "Quantitative Detection of siRNA and Single-Stranded Oligonucleotides; Relationship between Uptake and Biological Activity of siRNA", performs a similar experiment successfully in mammalian cells in which detection of only one of the strands of duplex siRNA in mammalian cells is used to assess quantitatively the concentration of siRNA in the cells under study.

The Office further states that there is no example set forth in the specification of actually detecting SRMs in mammalian systems. This should not be necessary. The specification makes clear that the mechanism involving SRMs is ubiquitous among organisms; the fact that this has first been detected in plants, along with a statement such as that on page 3, lines 10-13, that this is common to other organisms, absent evidence to the contrary, should be sufficient. Indeed, evidence of occurrence of SRMs as indicators of silencing in an organism other than a plant is demonstrated in working (not prophetic) Example 2. As evidenced by the references provided with this response, the empirical evidence made available in the field demonstrates that the methodology disclosed and claimed herein is an accurate statement of reality and thus enabled, functional and of importance.

In any event, the claims now require confirmation of silencing of the target gene.

The Office quotes from page 27 at lines 24-29 to the effect that the "precise role of 25 nt RNA and PTGS remains to be determined..." It is unclear why this would suggest that detection of SRMs in mammals would not be an indicator of silencing in such organisms. What this quote appears to be saying is that the mechanism by which these short RNA's act is not yet known, and it is at least in part because of this that the present claims are herein amended to delete "posttranscriptional." As noted above, this merely goes to the mechanism by which silencing occurs, not to the prognostic power of the claimed invention whereby detection of the SRMs

indicates that silencing, by whatever mechanism, is occurring. And this statement follows the direct experimental demonstration of the correlation of silencing with the presence of SRMs described above.

The Office then speculates that the claims "read on the detection of any sense or antisense 20-30 mer degradation product which would not be indicative of PTGS." There is no evidence of record that 20-30 mer degradation products of uniform length that are not indicative of silencing has ever been detected. In fact, one would clearly expect any such degradation products to be of random lengths.

The Office goes on to say that "to practice the claimed invention one of skill in the art would have to *de novo* determine which SARMs with what type and level of identity or similarity to the target gene would act as determinants for PTGS within the instant method." This is not understood. All one would have to do is to extract RNA from mammalian cells and determine the presence or absence of SRMs, (SSRMs and/or SARMs), and characterize them. No *a priori* predetermination would be required at all.

Conclusion

Applicants again express their appreciation to Examiners Schultz and Bowman for the telephone interview on 27 July, and in particular for the very helpful suggestion for amendment to the claims that appears to place them in a condition for allowance.

The specification teaches that the SRMs of uniform length detectable and exclusively associated with silencing in plants are also detectable in other organisms when silencing occurs. This has been demonstrated definitively in the application itself for plants and for nematodes. No reason has been shown to doubt the teaching in the specification that SARMs and/or SSRMs of

uniform length would be detectable only in those organisms (including, for example, mammalian systems) undergoing silencing and that these sequences would identify the gene being silenced as well as portions of such genes which would be useful to induce silencing.

Any doubt that the SRMs are indicative of a particular target gene having been silenced has been removed by the amendment to claim 32 that the silencing of the target gene be confirmed. Only if it is confirmed will it be considered that gene silencing has in fact been detected. This eliminates all uncertainty. As to proposed claim 82, the requirement for identity of the sequence with the silenced gene also adds a measure of certainty. As stated above, should the presence of claims 82-83 be the sole basis for issuing other than a Notice of Allowance, cancellation of these claims by Examiner's amendment is requested.

With regard to other particulars of the written rejection, applied to the claims prior to the present amendment, the citation of Elbashir as showing that duplexed RNA is an active species in silencing has nothing to do with the ability to utilize each strand separately or together to confirm that silencing is occurring and to identify genes that are undergoing silencing and sequences useful to induce the effect. The degree of similarity is reasonably left to the judgment of the practitioner and in any event, claims 68, 69 and 82 are free of this concern. Further, as evidenced by the issuance of the parent application of claims, encompassing identical and similar sequences in either SSRMs or SARMs, in which silencing is detected in plants into which exogenous nucleic acids have been introduced to induce silencing, this is an element of the invention which the U.S. PTO has already seen and found to be enabled. Importantly, the precision of degree of similarity required is now moot in light of the amendment to the claims which requires confirmation of silencing.

Finally, the Office appeared to assume in the outstanding rejection that what is taught by the application would have to be independently discovered in mammalian systems. This is not the case. The specification clearly states that the association between SRMs and silencing is applicable to organisms generally, including mammals. There is no need to identify any particular gene or any particular mammal or any particular SRM. Either the SRMs are there or they are not. If they are there, silencing is going on, and what they are is routinely determined by comparison to the genome of the organism in which silencing is occurring. Importantly, in the amended claim 32 and its dependent claims and in new claim 83, silencing or non-silencing of a particular gene is required to be confirmed.

Accordingly, enablement of the full scope of claim 32 and claims dependent thereon has been demonstrated and rejoinder of claims 33-34 is therefore proper along with allowance then, of claims 32-37, 41, 49, 66-83. Passage of these claims to issue is respectfully requested.

Should minor issues remain that could be resolved over the phone, a telephone call to the undersigned is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 616292000110.

Respectfully submitted,

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